ISSN: 2640-1223

Journal of Veterinary Medicine and Animal Sciences

Open Access | Research Article

Adaptation of Fowl Pox Vaccine Strain on DF-1 Cell and Evaluation of developed vaccine Immunogenicity and Efficacy in Ethiopia

Habtamu Demisse*

Hawassa University, College of Veterinary Medicine, Ethiopia.

***Corresponding Author(s): Habtamu Demisse**

Hawassa university, College of Veterinary Medicine

PO. BOX: 05, Ethiopia.

Tel: +251916377950; Email: dehabtamu@gmail.com

Received: June 03 2026

Accepted: June 22, 2026

Published Online: June 29, 2026

Journal: Journal of Veterinary Medicine and Animal Sciences

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

Copyright: © Demisse H (2026). *This Article is distributed under the terms of Creative Commons Attribution 4.0 International License*

Keywords: Antibody, DF-1 cell, Efficacy tests, Fowl pox virus, Immunogenicity.

Abbreviations: CAMs: Chorioallantoic Membranes; CFC: Chicken Fibro Blast Cell; DMEM: Dulbecco Modified Essential Medium; DNA: Deoxyribonucleic Acid; DPV: Day Post Vaccination; ELISA: Enzyme Linked Immune Sorbent Assay; FPV: Fowl Pox Virus; Kbp: Kilo Base Pairs; NVI: National Veterinary Institute; OD: Optically Density; OIE: Office of International des Epizootics; PBS: Phosphate Buffered Saline; PCR: Polymerase Chain Reaction; PI: Post Inoculation; SPF: Specific Pathogen Free; TCID: Tissue Culture Infectious Dose; VNT: Virus Neutralization Test.

Abstract

Fowl pox disease has a world-wide distribution and economic importance in poultry industry. Vaccination is the one of the best prevention approach for the disease. In Ethiopia, this vaccine was produced using chicken embryo fibro blast cell by tedious production system with high cost. The experimental study design was conducted at National Veterinary Institute for adaptation of DF-1 cell line with Fowl Pox Virus vaccine strain and then assessment of immunogenicity, safety and efficacy of the adapted vaccine was determined under study. The vaccinal strain adapted on DF-1 cell showed cytopathic effect within each passage level. The infectivity rate of Fowl Pox Virus vaccine working seed on study DF-1 cell on passage 7 was $log10^{6.3}$ TCID50/ml. The amplified product of DF-1 cell adapted had 578 base pairs as PCR result of fowl pox virus. DF-1 cell adapted fowl pox vaccine from passage 5 and 7 were inoculate into 5-week chickens and developed antibody at day 7 post inoculation. The boosting of adapted vaccine from passage 7 at day 14 in one group produced maximum antibody response with statistical significance ($p<0.0001$). The efficacy test was done on vaccinated and control group chickens with challenging virulent fowl pox virus isolate and the adapted vaccine showed 90% and 95% protection in single shot group and boosting group respectively while control group developed 95% of fowl pox lesion. The results obtained, therefore, suggest that DF-1 cell adapted fowl pox vaccine production method could be good production option for producing fowl pox virus vaccine for the prevention of the disease in poultry.

Background

Fowl Pox Virus (FPV) is a disease of chickens and other birds caused by a DNA virus of the genus avipoxvirus of the family poxviridae [1]. These diseases exit as cutaneous or diphtheritic form on the chickens and can commonly cause drops in egg production or retarded growth in younger birds [2]. Fowl pox disease has a world-wide distribution and its incidence is variable in different areas because of differences in climate, management and practice of regular vaccination [3]. Fowl pox has been controlled in developed countries. However, it is still a problem in most developing countries including Ethiopia and result serious economic impact across the countries [4]. Prevention against FPV infection is conducted either through strict biosecurity measurement or by vaccination [5].

Vaccination against FPV involves using attenuated vaccine either propagated on Specific Pathogen Free (SPF) or cell culture

Cite this article: Demisse H. Adaptation of Fowl Pox Vaccine Strain on DF-1 Cell and Evaluation of Developed Vaccine Immunogenicity and Efficacy in Ethiopia. J Vet Med Animal Sci. 2026; 10(1): 1156.

[6]. In Ethiopia, FPV vaccine is produced at National Veterinary Institute (NVI) by using SPF eggs. SPF eggs are expensive and unavailable easily; hence those eggs are imported abroad from different SPF egg producing farms with foreign currency. Additionally, the SPF based vaccine production system is tedious in which removal of embryo with sterile condition and preparation of the Chicken embryo Fibro blast Cell (CFC) from SPF eggs need large number of human power and time this implies such production system is stressful laboratory activity. In contrast to SPF egg based FPV vaccine production, it is possible to produce FPV vaccine using cell culture of avian origin such as DF-1 cells in which the vaccine production is easiest operation and produced with appropriate cost and time.

Methods

Description of experimental site

This study was conducted at the National Veterinary Institute (NVI), Bishoftu town, Ethiopia. NVI is only veterinary vaccine and drug producing institution in the Ethiopia. The institution has reached the high level by using standard equipments and materials for vaccine production and vaccine related research and this is assured by international accrediting institution with certificate. The certificates are ISO/QMS 9001: 2008 Quality Management System (QMS) certified by an international accrediting company ALCUMUS/ISOQAR and it is dedicated for manufacturing and marketing of veterinary vaccines. It also certified by ISO/IEC 17025:2005 in research and development laboratory for serological tests; 3 ABC ELISA for foot and mouth disease, C-ELISA for contagious bovine and caprine pluropenomunia, RBPT for brucellosis and ELISA test for peste des petits ruminants by Ethiopian National Accreditation Office (ENAO) [7].

Study design

Experimental study design was conducted on adaption of DF-1 cell with FPV vaccine working seed then safety, immune response test and efficacy test of adapted vaccine was examined at NVI, Ethiopia. The adaption of DF-1 cell with FPV vaccine was checked by observing Cytopathic Effect (CPE) after infecting the study cell with FPV vaccine working seed. The infectivity rate of FPV on study DF-1 cell was expressed by titration test. The further identity test for the conformation of DF-1 adapted FPV vaccine was ensured by PCR.

Complete Random Design (CRD) method was employed for total of 80 experimental chickens to divide into four groups. Each chick was randomly assigned to one of the 4 pens and identified using wing tag. Accordingly, a total of 80 chickens were assigned to 4 pens with 20 chicks per pen. Group A and B were planned for immunization of DF-1 cell adapted FPV vaccine from passage 5 and 7 respectively and Group B was boosted at day 14 again with passage 7. Group C was designed for vaccination of commercial Chicken embryo Fibro blast Cell (CFC) based FPV vaccine produced by NVI for comparison of efficacy and immune response with DF-1 cell adapted FPV vaccine while Group D was keep as control. Blood samples for immune response test was collected from each group before vaccination and post to vaccination at day 7, 14, 21, and 28 and subjected to indirect in house made ELISA kit for FPV antibody determination. All experimental and control group chickens were observed for takes and safety evolution and result were recorded. Additionally, all groups were challenged with field virulent isolate of FPV. Challenged groups were kept for 3 weeks and all observed outcomes were recorded.

Study methods

Experimental chickens hatching and management

The chickens were required for entire of this experiment were hatched artificially from SPF eggs at the NVI research and development department, hatchery section during the study time with aim to avoid maternal antibody interference during the study. Therefore, 130 fertile ten day incubated SPF eggs of lohmann breed were obtained from the NVI viral vaccine production department and those were used for hatching the experimental chickens. The fertility of obtained SPF eggs used for hatching was confirmed by candling. The process was carried out in a dark room using an electric candler. The candler was placed on the top of eggs for easy penetration of light through the eggs and the eggs were viewed with the light source. The fertile eggs were seen to be densely clouded with network of veins and containing the developing embryo. After candling process, the confirmed SPF fertile embryonated eggs were kept for eleven days in artificial incubator (super hatch, South Africa) with ideal temperature of 37.5^{0}C and ideal humidity of 50-65 % until hatching at NVI research and development department of egg hatchery section. After 11 days of incubation the hatchability percent of the eggs were expressed using the following formula [8].

$$\text{Hatchability (\%)} = \frac{\text{Number of chicks hatched } \times 100}{\text{Number of fertile eggs after candling}}$$

Then the hatched chickens were collected from incubator after 24 hour drying in the hatchery section and floor reared on the experimental room based on the study desire. The chickens feed on balanced commercial poultry ration with water provided as ad libitum and kept under strict hygienic measures throughout for aimed experimental study desire.

Sub culturing of DF-1 cells

The components used for sub culturing of study cell line like PBS (phosphate buffered solution), 0.25% trypsin and growth medium were warmed at 37°C in water bath (Clifton, England). DF-1 cell placed at 75cm^2 flask was taken from 5% CO2 incubator (Thermo scientific, USA) and examined under inverted microscope (Motic, Hong Kong) for the development of a complete monolayer. The flask containing DF-1 cells were then placed under class-II safety cabinet (ESCO, Singapore) and the growth medium overlaying the cell monolayer was removed and washed three times by PBS. The confluent monolayer was rinsed with 2 ml of sterile 0.25% trypsin for about 5 minutes then existing trypsin was removed and observed under an inverted microscope for cell layer dispersing and incubated for 5 minutes in 5%CO2 incubator. The dispersed cell was gently pipetted with adding 8 ml of complete growth medium. The viability of cells was checked with trypan blue stain by using of automatic cell counter machine (EVE, Chain). The viable cells counted were 1.2x10^6/ml (86% is viable out of 1.4x10^6/ml cell) then 40ml of complete growth medium was added and a cell suspended in the flask was spitted into two 25cm^2 tissue culture flasks. These flasks were placed horizontally in the 5% of CO2 incubator at 37 ºC. The flasks were examined under the inverted microscope for the formation of complete monolayer with spindle fibroblast morphology after 24 hours of incubation [9].

Inoculation of FPV vaccine working seed into DF-1 cells

The PBS and maintenance media were warmed at 37°C water bath for this activity. The biological safety cabinet II (ESCO,

Chain) was disinfected with 70% ethanol alcohol for virus inoculation activity. Confluent monolayers of DF-1 cells within two 25 cm^2 flasks were transferred to safety cabinet II after 24 hours of incubation. The growth mediums in the flask discarded and the monolayer was washed with PBS three times. The DF-1 cells were then infected by adding 500 µl of FPV vaccine strain working seed with lot number lot number 2/21 and titer of $log10^{5.5}$ TCID50/ml which obtained from NVI quality assurance department, vaccine seed preparation section. Then followed with circular rotation and incubated at 37°C for 1 hour for adsorption. Following incubation for 1 hour, the virus inoculate was discarded and 10ml of DMEM maintenance media was added into an infected flask. The second flask containing confluent monolayer cells with growth medium discarded and was washed with PBS three times and filled with maintenance medium only and kept as control. Then all flasks were placed into 5% CO2 incubator at 37°C and observed under inverted microscope for CPE formation for the next six consecutive days. The maintenance medium with in the flasks was changed after three days of incubation. After development of marked CPE, the infected cells in with in flask were harvested and labeled by indicating passage number and stored at -20°C. The harvested virus was freeze–thawed three times and inoculated further to fresh confluent monolayer of DF-1 cells using the same technique and observed for CPE appearance for six days. This process were repeatedly done up to passage 7 and stored at the -20°C until next activity were conducted [10].

Titration of FPV Vaccine strain Propagated on DF-1 cells

The FPV vaccine propagated on DF-1 cell to be titrated were diluted serially in 10 sterile universal bottles with 500 µl of DF-1 adapted viral suspension from each of passages (passage 1 up to passage 7 separately) within 4.5 ml of basal DMEM medium. The 96 micro plates (Jet Biofilm, chain) wells were filled with 100 µl viral dilutions form each universal bottle and 100 µl DF-1 cells respectively up to column 10 and column 11 was left empty. Column 12 was inoculated with DF-1cell as control. The micro plate wells were labeled and sealed by micro plate sealer and incubated at 37°C within 5% CO_2 incubator for 6 days and observed under inverted microscope for CPE formation up to the last incubation period. The formula stated by Reed and Muench [11], was used to determine the titres assay for each passages.

Log10 TCID 50% = - (-log10 of dilution showing a positive monolayer's next above 50% - (difference of logarithms × logarithm of dilution factor).

Where; Difference of logarithms calculated as;

$$\text{Difference of logarithms} = \frac{(\%\ \text{infected at dilution immediately above } 50\%) - 50\%}{(\%\ \text{infected at dilution above } 50\%) - (\%\ \text{infected at dilution below } 50\%)}$$

Where; TCID: Tissue culture infectious dose.

Molecular Identification of DF-1 cell Adapted FPV Vaccine

DNA extraction

The DNA of DF-1 cell adapted FPV vaccine was extracted using Qiagen™ Mini columns (Qiagen, Germany). 20 µl of Qiagen Protease was pipetted into the bottom of each labeled 1.5 ml of microcentrifuge tube. 200 µl of suspected sample and 200µl of lysis buffer AL was added for each tube and mixed by vortexing for 15 second and incubated at 56ºC (Labnet, Chain) for 10 minutes. 200 µl of 96% ethanol was added to the sample tube and mixed again by vortexing. The mixture was carefully transferred to a labeled mini spin column placed in a 2 ml collection tube and centrifuged for 1 minute at 8000 rpm. The collection tube was removed and replaced by new one, and then 500 µl of wash buffer AW1 was added into the spin column and centrifuged for 1 minute at 8000 rpm. The collection tube was again changed by new tube and spin column was opened and 500µl of wash buffer AW2 was added and centrifuged for 3 minutes at 14000 rpm. These was followed with placing the spin column in a clean 1.5 ml microfuge tube and the spin column was opened and 200 µl of elution buffer was added and incubated at room temperature for 5min, and then centrifuged at 8000 rpm for 1 minute to elude the DNA into the microfuge tube. The nucleic acid bound to the silica membrane was eluted and the tube was labeled properly. The extracted DNA was used as template for the next activity.

Polymerase chain reaction for FPV

FPV based PCR was performed using a primer pair described by Lee and Lee (1997). The sequences of the primer (VBC-biotech, Austria) were (F: 5'– CAGCAGGTGCTAAACAACAA-3') and (R: 5'-CGGTAGCTTAACGCCGAATA-3'). The primers used were based on FPV 4b gene sequence. The size of the amplified DNA fragment of FPV using these two primers is expected to be 578 base pair (bp) in length [12].

The PCR reaction was performed with each of 12 µl of IQ super mix (Bio-Rad, USA), 2.5 µl of forward primer, 2.5 µl of reverse primer, 3 µl of RNase free water and 5 µl of template DNA. PCR tube containing 25 µl of final volume was placed in a thermal cycler (Applied Bio system, Singapore) through protocol initial denaturation at 94°C for 5 min. Then it followed by with denaturation at 94°C for 1 min, annealing at 60°C for 1 min and elongation at 72°C for 1 min for 30 cycles. The final elongation was done at 72°C for 2 min.

Agarose gel electrophoresis of PCR product

The amplified products of PCR were separated by agarose gel electrophoresis using 1.2% agarose gel [13]. Briefly, 1.2 g of agarose was added into a flask containing 100 ml of 1X TAE (Tris-acetate-EDTA) buffer. The mixture was boiled with micro-oven (BOSCH, Germany) for 1 minute to dissolve and allowed to cool. The 5µl of intercalating dye (Hi media, India) was added to the flask containing the agarose solution. The gel was poured on gel caster dish placed horizontally. The comb was placed in the caster dish and waited for 20 minutes until the gel was solidified and the comb was removed after the given time. Then gel was placed in the electrophoresis tank containing 1X TAE buffer.

In the first and last lane 10 µl 100 bp DNA marker (Hi media, India) was added and while on other lanes 10 µl of sample PCR products, negative and positive control was added containing 4 µl of loading dye in PCR products. Then the electrophoresis (Kodak Biomax, USA) was run for 1 hour at 120V and PCR products were observed under the UV trans-illuminator and photographed (UniTec, France). Finally, the band size of PCR products were recorded and compared with standards (578 bp).

Immune response test of DF-1 cell adapted FPV vaccine

Eighty chickens with age of five week which were hatched at NVI during this study time were used for immune response, safety, takes and efficacy tests. The chickens divided into four groups each group with twenty chickens and named as group A, B, C and D. The first three groups (A, B and C) were used as experimental group while group D was used as a control group according to OIE (2018) protocol. Groups A and B were vaccinated

with 0.02ml of DF-1 adapted FPV vaccine from passage 5 and passage 7 respectively. Group C was vaccinated with 0.02ml of CFC based live FPV vaccine which was prepared at NVI for commercial purpose. Group D was vaccinated with 0.02 ml uninfected DF-1 cell suspension to serve as negative control. The all groups were vaccinated by wing web puncture administration route. The test group B was boosted on the left wing opposite to initial vaccination site after 2 weeks with the same FPV vaccines given earlier for this group [14].

The blood samples for serum were collected from all groups of chickens from their wing vein before vaccination and post to vaccination at day 7, 14, 21 and 28 by using 3ml sterile disposable syringe with 22GX11/4. Finally, the serum collected at 1.8ml cryo vial, labeled and stored at -20ºC for determining antibody level in different group of chickens using in house made FPV ELISA kit developed at NVI according to OIE protocol. Hence, a commercial ELISA kit for FPV is not available at the market. Therefore, OIE recommended testing immune response of large number of serum by in house made ELISA following its declared protocol.

Takes and safety tests

Takes test was done in all vaccinated chickens groups which obtained the vaccine as single or boost shot earlier in immune response test were observed and recorded for 7 to 10 days for the 'takes' lesions at the vaccinated site, which was indication for successful vaccination. In addition, the earlier vaccinated experimental groups were also observed for adverse effect or any healthy complications post to vaccination then the consequence was observed and recorded to determine safety issues [15].

Efficacy test

This test was conducted by inoculating field isolate virulent FPV in to chickens group vaccinated earlier with DF-1 cell Adapted FPV vaccine (from passage 5 and 7) and CFC based FPV vaccine. The control group was also inoculated with the virulent FPV isolate. The all challenged experimental and control group were observed for 3 weeks for gross FPV pathological lesions development across body of chickens after inoculation of field isolate FPV. Chicken groups vaccinated before with DF-1 adapted FPV vaccine, CFC based FPV vaccine and control group were challenged with FPV field isolate at 28 days post vaccination (after blood taken). The challenge virus with titer of $log10^{5.2}$ TCID50/ml isolated from previously confirmed positive case was inoculated by 0.02ml per chickens via wing web puncture. Then, the challenged chicken was followed 3 weeks for FPV lesion development and the results were recorded. The protection percentage of the vaccine was determined as percentage of the chickens remains free from the FPV lesions development from the total number of challenged chickens per group [16].

Ethical clearance

The study was approved by NVI research ethical review committee ref. no. 156/ N4 10/09/2022(Appendix 1).

Data management and analysis

The collected data during laboratory investigation was entered into Microsoft office excel spread sheet 2010. Descriptive and inferential statistics was used to summarize laboratory findings. PCR product of 578 bp for FPV band size on agarose gel electrophoresis was used for DNA detection determination. Figure drawing and data analysis was also performed by using Graph Pad Prism software 9.3.1. Significance of difference between experimental groups was determined by analysis of variance (one way ANOVA) test.

Results

Hatchability of experimental chicken

The chickens required for entire experimental activity were obtained after 11 day of artificial incubation. The hatchability percent of the fertile eggs was 73% as indicated in table 1. The coat colors of the hatched chickens were brown, white and mixed (Figure 1).

Table 1: Hatchability percent of fertile eggs in incubator.

Item	Number (%)
Fertile eggs	130
Hatchability percent	95(73%)
Day of incubation	11

Figure 1: Experimental chickens hatched at incubator.

Adaption of FPV on DF-1 cell

Following six days of incubation the DF-1 cell adapted with FPV vaccine working seed strain had minor CPE development (floating cell) was observed on passage 1. On the passage 2, some advance CPE with floating cell observed when compared to passage 1 at end of incubation date. However, CPE development from passage 3 up to 7 is good, about 60% up to 80% CPE was observed on the infected flasks. The CPE effect was characterized by floating of cells at 3rd day of Post Inoculation (PI). Rounding of cells, aggregation of cell and sloughing of cells in flask were examined under inverted microscope at 4 up to 6 days PI. No CPE effect was observed on negative control monolayer. The CPE effect of infected and control DF-1 cell monolayer were indicated below (Figure 2).

Titration of the FPV vaccine adapted on DF-1 Cell

The titer of DF-1 adapted FPV vaccine strain was determined according to Reed and Muench (1938). The infectivity of DF-1 adapted FPV vaccine strain on passage 1, passage 5 and passage 7 was log $10^{4.5}$ TCID50/ml, log $10^{5.5}$ TCID50/ml and $log10^{6.3}$ TCID50/ml respectively as indicated on Table 2.

PCR test result

The DF-1 adapted FPV vaccinal strains from passage 1 up to 7 were subjected for conventional PCR for identity test confirmation. An amplified product of 578 bp (Figure 3) was detected on agarose gel electrophoresis from passage 1 up to passage 7.

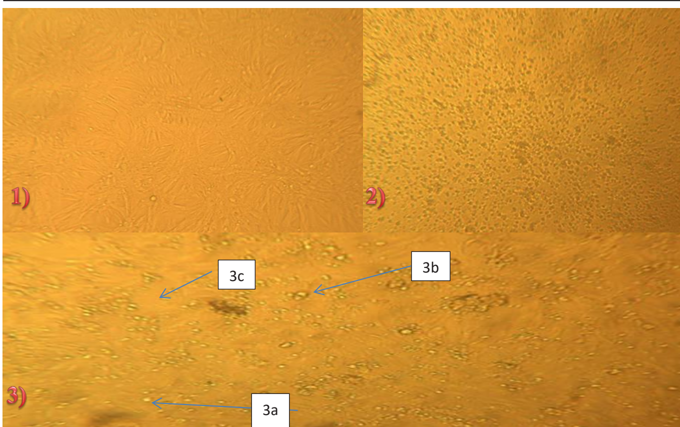


Figure 2: 1) DF-1 cell control (×10). **2)** CPE (floating of cells) developed by DF-1 adapted FPV vaccine strain on passage 5 at 3rd day of PI (×10). **3)** CPE (rounding of cells(**3a**), aggregation of cell (**3b**) and sloughing of cell (**3c**)) developed by DF-1 adapted FPV vaccine strain on passage 7 at 5th day of PI (×10).

Table 2: Titration result DF-1 cell adapted FPV vaccine at different passages.

Passage	Titer (log_{10}TCID50/ml)
1	$10^{4.5}$/m1
2	$10^{4.7}$/m1
3	$10^{4.8}$/m1
4	$10^{5.3}$/m1
5	$10^{5.5}$/m1
6	$10^{5.8}$/m1
7	$10^{6.3}$/m1

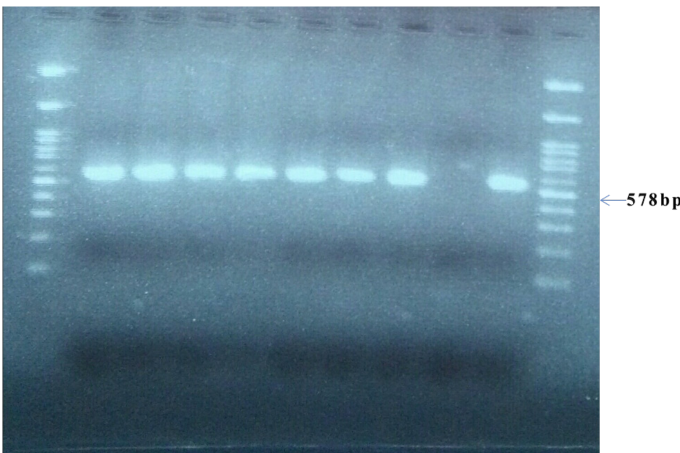


Figure 3: PCR analysis of FPV 4b gene showing amplified 578 bp fragments. Where; lane 1: Infected DF-1 cell of passage number 1; lane 2: Infected DF-1 cell of passage number 2; lane 3: Infected DF-1 cell of passage number 3; lane 4: Infected DF-1 cell of passage number 4; lane 5: Infected DF-1 cell of passage number 5; lane 6: Infected DF-1 cell of passage number 6; lane 7: Infected DF-1 cell of passage number 7; lane 8: Negative control; lane 9: Known positive control; lane M: Molecular marker with 100bp.

Takes and safety test result

At the 7th Day Post Vaccination (DPV) the vaccinated right wings of all chickens were examined for the presence of takes; (18 chickens from group A, 17 chickens from group B (before boosting) and 17 chickens from group C respectively has inflammation at 7th day) as shown in Table 3. The remaining birds appeared normally. The number of chickens with inflammation increased to (19, 19 and 18) in group A, group B and group C respectively at the 10th DPV and had developed small circular lesions with mild swelling on the right wing (Figure 4). The group B after boosting showed 19 takes at the 7th and 10th DPV. Any sign of complication or death was not observed after immunization DF-1 adapted vaccine to experimental chickens in each group.

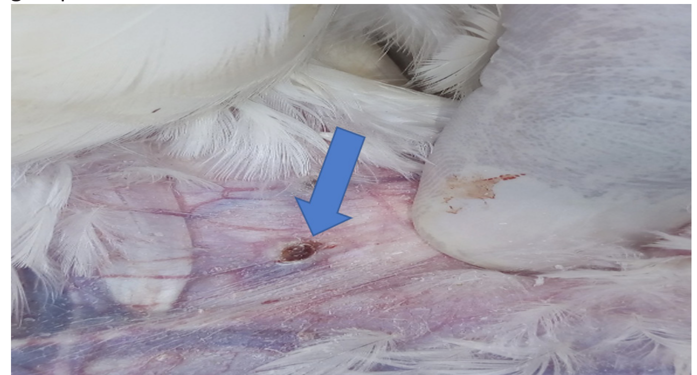


Figure 4: "Takes" image at 10 days post-vaccination.

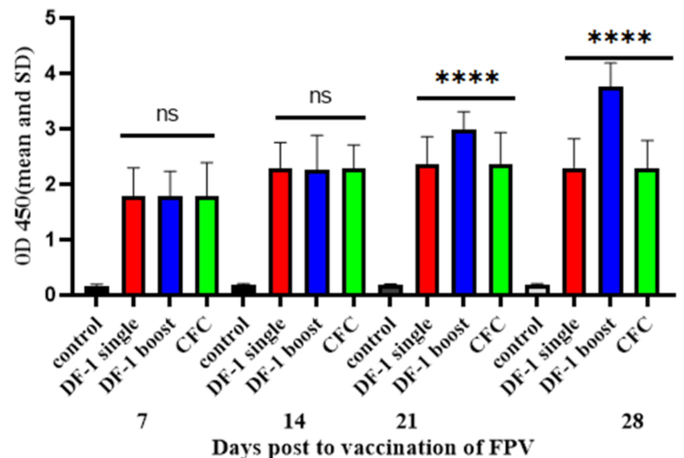
Table 3: Post vaccinal takes and safety evaluation of vaccinated and control groups.

Study groups	Vaccine type used	Number (%)		Safety issue	
		7 DPV	10 DPV	NC	ND
Group A	DF-1 adapted of passage 5	18(90%)	19(95%)	0	0
Group B single	DF-1 adapted of passage 7	17(85%)	19(95%)	0	0
Group B boost	DF-1 adapted of passage 7	19(95%)	19(95%)	0	0
Group C	NVI CFC	17(85%)	18(90%)	0	0
Group D	DF-1cell only	0	0	0	0

Where; **DVP**: Day Post Vaccination, **NC**: Number showed complication and **ND**: Number of death.

Immune response results

All experimental groups chickens vaccinated with DF-1 adapted FPV vaccine were developed antibody against the vaccine. The ELISA antibody response was detectable as early as day 7 Post Inoculation (PI) in vaccinated chickens. The antibody to FPV was present up to the end of the experiments (day 28 PI) in all vaccinated experimental groups chickens. The maximum antibody response in these chickens occurred between day 14 and 28 PI. The antibody response in boosted group of chickens showed increment at day 21 and 28 PI.



Where; **ns**: Not significant, ****: p <0.0001.

Figure 5: Immune response result of chickens vaccinated with DF-1 adapted and CFC based FPV vaccine at day 7, 14, 21 and 28 PI.

Efficacy test result

Following the challenging of all groups with virulent FPV isolates for 3 weeks, 95% of unvaccinated control group chickens had developed FPV lesion across the comb and wattles as shown in figure 5. The chicken groups vaccinated with commercial CFC based FPV vaccine produced in NVI showed 10% of FPV lesion. However, the chickens' vaccinated with DF-cell adapted FPV vaccines from passage number 5 and 7 showed 10% and 5 % FPV lesion across comb and wattles with in single and boost groups respectively as indicated in figure 7.

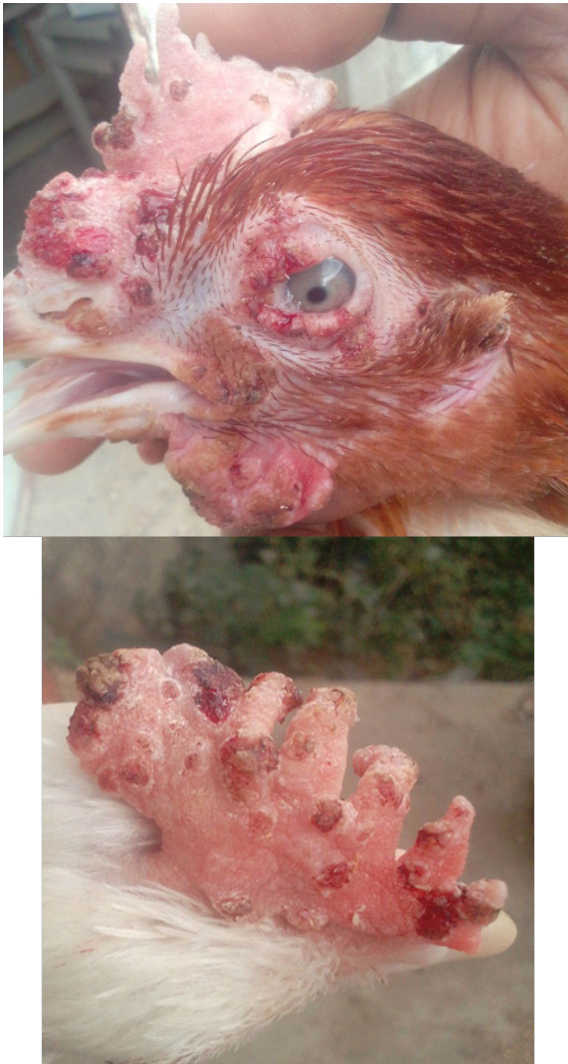


Figure 6: FPV lesion developed on comb and wattles of control group chickens.

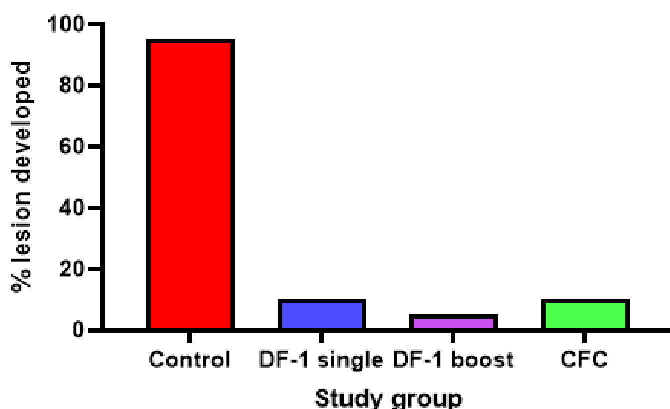


Figure 7: Percentage of FPV lesion developed on vaccinated and control groups.

Discussions

The present study was designed for adaption of FPV vaccine strain on the DF-1 cell and also it determined the immunogenicity, safety and efficacy of the adapted vaccine.

In this study the experimental chickens used for determination of immunogenicity, safety, efficacy in experimental group and as well as used as control group were hatched artificially at the NVI research and development department of egg hatchery section and during hatchery activity 95 (73%) chickens were hatched from the total of 130 SPF fertile embryonated eggs after 11 days of artificial incubation. The current finding was similar with early reports stated artificially incubated chickens' hatchability was in range from 65-75% [17].

The slight floating cell CPE characters was observed on passage 1 during the last day of incubation then, after harvesting the cell and refreshing to the next flask the advance CPE development observed with floating of cells (starting from passage 3) at 3rd day of Post Inoculation (PI) as well as rounding of cells, aggregation of cell and sloughing of cells were examined under infected flask from passage number 3 up to passage number 7 from 4 - 6 days PI. Similar findings CPE effect to current study were also reported on FPV adapted on Chicken Fibroblast Cell (CFC) showed with rounding of cell and massive sloughing of cells at 5th day of PI [18]. The CPE characters following the sub culturing of the study cell line of this study also in line with cellular changes observed on FPV adapted on Baby Grivet Monkey kidney (BGM) cell and in which rounding of cell, clumping of cell and degeneration of cell observed [19].

The infectivity of DF-1 cell adapted FPV varies with in each passage level. The titer of the virus was start increment from passage 1 to passage 7 as indicated on Table 3. The highest titer value was $10^{6.3}$ TCID50/ml at passage level 7. These findings were closely correspondent to the infectivity of FPV vaccinal strain propagated on ducks embryo Fibroblast cell was $\log10^{6.67}$ TCID50 /ml at passage level 7 [20]. The present study also related with finding on the propagation of FPV on DF-1 cell and found that DF-1 cell were permissive for FPV replication and showed there was significant infectivity titer at the propagation [21].

The PCR analysis outcome of this study was in line with stated agarose gel band for FPV vaccine using specific 4b gene was 578bp [22]. The PCR finding of the present study was also similar with the findings of PCR amplification of 4b gene with 578 bp were positive for FPV by PCR assay [21]. In addition, this study finding was in agreement with the pervious finding of the agarose gel band result of FPV with 4b gene was 578 bp [23].

The takes test of this study showed similar result with takes test was detected at the FPV vaccine applied site (90-100%) at day 7th and 10th day post vaccination [24]. The result of this study also in line with result reported that live FPV vaccination developed (80-95%) small circular lesions on the wing on the 7th and 10th day post vaccination [25]. All vaccinated chickens remained healthy and active as the unvaccinated controls. This result is similar to the finding of the safety test of the FPV vaccine in which there is no obvious clinical signs were detected among all vaccinated chickens post to vaccination [26]. The result of this study also agreed with the description given by about safety issue of live FPV vaccine in poultry [27].

Immunogenicity test result of these findings was in accordance to previous mean antibody at day 14 post infection of

FPV vaccine reports in which it showed a gradual increase of antibody values in all inoculated experimental groups [28]. There was significance difference (p <0.0001) of mean of antibody in boosted group at day 21 and 28 post inoculation. This result was in agreement with the previous findings reported the level of antibody produced against the FPV raised after boosting with significant variation compared to other groups [29].

In the current studies the result of the vaccine efficacy studies indicated that DF-1 adapted FPV vaccine were protective against challenge with virulent field isolate FPV. The chicken vaccinated with DF-1 adapted FPV vaccine strain from passage 5 and passage 7(boosted group) produced 90% and 95% of protection respectively while commercial NVI CFC based vaccine produced 90% of protection against the challenge field isolate virus. The control groups responded to challenge virus and developed FPV clinical manifestation lesion across the comb, wattles and non-feathered part of chickens' body. The results of this study agree with OIE, (2018) who reported that 90% of challenged non-vaccinated control birds should show characteristic FPV lesions and at least 90% of vaccinated experimental group birds should remain normal without evidence of any FPV lesions.

Conclusion

Based on the data presented in this study the developed DF-1 cell adapted FPV vaccine was safe and protective as NVI commercial chicken fibroblast cells-based vaccine produced using SPF eggs. The adapted vaccine has good CPE and infectivity titer at each passage level. The propagated vaccinal strain on the study cell has also the same molecular identity as FPV. The DF-1 adapted FPV vaccine was found to be safe as no abnormal signs or mortality was observed during safety test throughout the monitoring period. The immunogenicity of adapted vaccine is similar to commercial vaccine while the boosting of adapted vaccine has also good protection at given evaluation period. The adapted vaccine strain at passage 5 and 7 showed similar efficacious as CFC based prepared FPV vaccine against field strain FPV. The results obtained, therefore, suggest that DF-1 adapted FPV vaccine production technique could be good production option for producing FPV vaccine for the prevention of the disease in poultry.

Author declarations

Acknowledgements

First and for most, blessed be my heavenly father, God and his mother, saint mary, for help, leading and strengthening in every moment of my life.

I owe my deepest gratitude to my advisor, Dr. Mishamo Sulayeman for his intellectual advice support, and correcting of this manuscript.

I am highly grateful to my co advisor, Dr. Belayneh Getachew who allowed me to work under this project with close supervision and for his overall laboratory activity guidance.

My special thanks also go to my co advisor, Dr. Nebyou Moje for his valuable advice and constructive comments.

I am deeply indebted to the National Veterinary Institute staffs particularly to, Dr. Takela Abyenh, Dr. Takela Tsegra, Ms. Hawa Mohammed, Ms. Wubate Woldamedhin, Ms. Aresema, Mr. Kedire Shirfa, Mr. Eyob Asefa, Mr. Kasaye Adamu, Mr. Abinate, Mr. Getawu, Mr. Kenawu and all National Veterinary Institute staffs for their support during laboratory works.

I am also grateful to Addis Abeba University, College of Veterinary Medicine and Agriculture for providing of dormitory service for me throughout study time.

Furthermore, I like to thank Mareka woreda Administration for giving sponsorship for my MSc program.

Finally, it is also my pleasure to extend my enthusiastic thankful expression to my beloved father Demisse Kasa, my beloved mother Ayenalem Silashe and to all my respected family for encouragement, financial support and overall assistance in my life.

References

1. MacLachlan N, Dubovi E. Poxviridae. 4th ed. London; Boston: Academic Press. 2011: 157-160.
2. Black A, Soncini R, Ruthes O, Madureira S, Flores R. An atypical fowl pox outbreak in broiler in Southern Brazil. Avian Dis. 1995; 39: 902-906.
3. Tripathy N, Reed M, Swayne D. Diseases of poultry. In: Saif YM, Barnes HJ, Glisson JR, Fadly AM, McDougald LR, Swayne DE, editors. Diseases of Poultry. USA: Iowa State University Press; 2013: 333-349.
4. Hana T, Senait B, Benti D. Evaluation of the safety and efficacy of combined Newcastle disease, fowl pox and fowl typhoid vaccine under laboratory condition. J Vet Med Anim Health. 2018; 10: 60-66.
5. Njue S, Kasiiti J, Macharia J, Gacheru S, Mbugua H. Health and management improvements of family poultry production in Africa – survey results from Kenya. In: Characteristics and parameters of family poultry production in Africa. IAEA. 2002; 1: 39-45.
6. Dasgupta A, Hammarlund E, Slifka K, Früh K. Cowpox virus evades CTL recognition and inhibits the intracellular transport of MHC class I molecules. J Immunol. 2007; 178: 1654-1656.
7. NVI. Product catalogue for vaccine No. 2. 2016: 1-35.
8. Getachew B, Aberra M, Wondmneh E, Gebeyehu G, Tadelle D. Analysis of fertility, embryonic death, hatchability of artificial incubator used and egg quality of indigenous chickens' ecotypes in Gambella regional state Ethiopia. Int J Anim Sci. 2021; 7: 389-397.
9. ATCC. Animal Cell Culture Guide of UMNSAH/DF-1 cell CRL-12203. 2021. Available from: www.atcc.org.
10. Tripathy N, Schnitzlein W, Morris P, Janssen D, Zuba J, Massey G, Atkinson C. Characterization of poxviruses from forest birds in Hawaii. J Wildl Dis. 2000; 36: 225-230.
11. Reed J, Muench H. A simple method of estimating fifty percent end points. Am J Hyg. 1938; 27: 493-497.
12. Binns M, Boursnell M, Tomley F, Campbell J. Analysis of the fowl poxvirus gene encoding the 4b core polypeptide and demonstration that it possesses efficient promoter sequences. Virology. 1989; 170: 288-291.
13. Roy B, Joardar S, Samanta I, Das P, Halder A, Nandi S. Molecular characterization of fowl pox virus isolates from backyard poultry. Adv Anim Vet Sci. 2013; 1: 54-58.
14. Stathis G, Michael S. Spotlight on Avian Pathology: Fowl pox virus. Avian Pathol. 2018; 37: 54-89.
15. OIE. OIE Terrestrial Manual: Fowl pox. 2018: 906-913.
16. Sonaiya E, Swan S. Small-scale poultry production, technical guide manual. FAO Animal Production and Health. Rome: Food

and Agriculture Organization of the United Nations; 2004.

17. Yadav S, Dash B, Kataria M, Dhama K, Gupta S, Rahul S. Pathogenicity study of different avipoxviruses in embryonated chicken eggs and cell cultures. Indian J Vet Pathol. 2007; 31: 17-20.

18. Joshi R, Namital J. Growth and cytopathogenicity of a field isolate of fowl pox virus in BGM-70 cell line. Indian J Comp Microbiol. 2011; 32: 19-21.

19. Rebeka S, Nazmul H, Md Tanvir R, Shamim A, Mizanur R, Sabiha S, Bahanur R. Isolation and molecular detection of Fowl pox and Pigeon pox viruses for the development of live attenuated vaccine seeds from the local isolates. J Bangladesh Agric Univ. 2019; 17: 211-219.

20. Efstathios S, Giotis M, Skinner A. Spotlight on Avian Pathology: Fowl pox virus. Avian Pathol. 2018; 10: 1080-1085.

21. Ha H, Howe L, Alley M, Gartrell B. The phylogenetic analysis of avipoxvirus in New Zealand. Vet Microbiol. 2011; 150: 80-87.

22. Masola S, Mzula A, Tuntufye H, Kasanga C, Wambura P. Isolation and molecular biological characterization of fowl pox virus from specimen of cutaneous nodular lesions from chickens in several regions of Tanzania. Br Microbiol Res J. 2014; 4: 759-771.

23. Roy B, Joardar S, Samanta I, Das P, Halder A, Nandi S. Molecular characterization of fowl pox virus isolates from backyard poultry. Adv Anim Vet Sci. 2013; 1: 54-58.

24. El-Mahdy S, Christine M. Efficacy of fowl pox vaccines against Egyptian isolated strain during 2012. Vet World. 2014; 7: 656-660.

25. Hye Jeong H, Alley M, Howe L, Gartrell B. Evaluation of the pathogenicity of avipoxvirus strains isolated from wild birds in New Zealand and the efficacy of a fowl pox vaccine in passerines. Vet Microbiol. 2013; 16: 268-274.

26. Meseko C, Shittu I, Akinyede O. Seroprevalence of fowl pox antibody in indigenous chickens in Jos North and South council areas of Plateau State, Nigeria: implication for vector vaccine. Vet Sci. 2012; 15: 49-71.

27. Girish S, Barry A, Kersting GS. Field safety and efficacy of a unique live virus vaccine for controlling avian encephalomyelitis and fowl pox in poultry. Vet World. 2019; 12: 1291-1298.

28. Boyle B, Pye D, Coupar H. Comparison of field and vaccine strains of Australian fowl pox viruses. Arch Virol. 1997; 142: 737-748.

29. Niraj K, Sidduqe M, Hossian M, Islam M, Hyung J, Song H. Immune response and efficacy of pigeon pox virus vaccine and fowl pox virus vaccine in chickens. Korean J Vet Serv. 2007; 30: 497-503.

Appendix 1: Ethical clearance certificate

ብሔራዊ የእንስሳት ጤና ጥበቃ ኢንስቲትዩት
NATIONAL VETERINARY INSTITUTE

ቁጥር
Ref.No 156/ruu
ቀን
Date 10/9/2022

To: Habtamu Demissa

The Research Ethical committee of the national Veterinary Institute reviewed and discussed your research project entitled DEVELOPMENT OF DF-1 CELL ADAPTED FOWL POX VACCINE AND A HOUSEMADE ELISA KIT FOR DETECTION OF ANTIBODIES AGAINST FOWL POX VIRUS on January 12/2022. After discussion and review of your project, it is found scientifically and ethically sound from relevance, originality and technical competence point of view.

Hence, the project is allowed to be executed provided that:

1. All procedures and conditions stipulated in the proposal are respected and any deviations, variations or changes may be made only in consultation between and reported to the committee, any progress will be made with written agreement.
2. All comments given by the committee should be considered and fulfilled by the researchers.
3. The project activity is open for occasional supervision by the committee whenever this is deemed necessary.

The committee expects to be informed about he progress of the study with any changes in the protocol.

Yours sincerely,

በላይነህ ጌታቸው (ዶ/ር)
የምርምርና ስርፀት
ዳይሬክቶሬት ዳይሬክተር

Cc:

- Director General
- Deputy Director General

National Veterinary institute

✉ 19
ደብረ ዘይት ኢትዮጵያ
Debre Zeit ETHIOPIA

☎ 011 433 - 84 - 11/16
251-911-51-08-94
FAX: 011 433-93-00

E-mail: nvi-rt@ethionet.et
info@nvi.com.et
Website: www. nvi.com.et